

**PREVENTION OF CLASSICAL SWINE FEVER
IN THE BORDER REGION CROATIA – SERBIA
(STOP – CSF)**

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For the Publisher / Za izdavača

dr Dragica Stojanović, Senior Research Associate, Director of the Institute

Reviewers / Receptenti

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Dr Emiliya Ivanova

National Diagnostic and Research Veterinary Medical Institute Sofia, Bulgaria

Dr Tamaš Petrović

Scientific Veterinary Institute „Novi Sad“, Novi Sad, Serbia

Editor in Chief / Glavni i odgovorni urednik

Dr Tamaš Petrović, Senior Research Associate

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SPREČAVANJE ŠIRENJA KLASIČNE KUGE SVINJA U POGRANIČNOM REGIONU KROZ POBOLJŠANJE SANITARNIH STANDARDA I EDUKACIJU FARMERA (STOP – KKS)

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“PREVENTION OF CLASSICAL SWINE FEVER IN THE BORDER REGION CROATIA – SERBIA (STOP – CSF)” „SPREČAVANJE ŠIRENJA KLASIČNE KUGE SVINJA U POGRANIČNOM REGIONU HRVATSKA – SRBIJA (STOP – KKS)“

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CLASSICAL SWINE FEVER NATIONAL REFERENCE LABORATORY – ROLE AND IMPORTANCE –

Vesna Milicevic¹, Ljubisa Veljovic¹, Jelena Maksimovic Zoric¹, Dobriša Jakic-Dimic¹

1. Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia

Abstract

National reference laboratory for Classical Swine Fever was established 08.7.2009. by the Decision of Ministry of Agriculture, Trade, Forestry and Water Management of Republic of Serbia No 323-06-5309/2009-05 which defines conditions according to available facilities, space, equipment and professional staff, in line with Law on Veterinary Medicine, articles 21 and 27. Obligations of the National Reference Laboratory, defined by this decision, are laboratory diagnosis of classical swine fever on samples from domestic and wild animals, the reference laboratory testing, maintaining the reference sera and standard reagents, as well as classical swine fever virus isolates, the implementation of new diagnostic methods, testing and quality control of diagnostic reagents and vaccines, the organization of interlaboratory comparative testing at the national level as well as confirmatory testing on the samples obtained from other laboratories.

Key words: classical swine fever, National reference laboratory

Introduction

Institute of Veterinary Medicine of Serbia (IVMS) is the oldest veterinary institution, established in 1926. The main activities of the Institute are applied researches in biotechnic science, clinical and laboratory investigations, prevention, control and eradication of animal infectious diseases, quality control of animal feed and food and veterinary medicines.

According to the activities, within the Institute, there are department for food safety and department for health protection with virology, immunology, bacteriology, parasitology, pathology, epidemiology departments as well as, department for epidemiology and fish health protection, department for epidemiology and swine health protection, department for epidemiology and poultry health protection, department for epidemiology and ruminants/equine health protection and laboratory for BSE.

Institute of Veterinary Medicine of Serbia is national reference laboratory for classical swine fever, african swine fever, foot and mouth disease, swine vesicular disease, rabies, blue tongue disease, equine infectious anemia, equine influenza, glanders, dourine, bovine leukaemia, brucellosis, salmonellosis and fish diseases.

Reference laboratories play important role in the harmonisation of laboratory diagnostic tests and the standardisation of veterinary vaccines and the building confidence between international trading partners. These activities include advice and consultancy, training, research, disease surveillance, maintenance of

culture collections, evaluation of reference methods, preparation of reference materials and organisation of inter-laboratory comparisons (Edwards and Alexander, 1998).

National reference laboratory for Classical Swine Fever was established 08.7.2009. by the Decision of Ministry of Agriculture, Trade, Forestry and Water Management of Republic of Serbia No 323-06-5309/2009-05 which defines conditions according to available facilities, space, equipment and professional staff in line with Law on Veterinary Medicine, articles 21 and 27.

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In National reference laboratory, there are 3 doctors of veterinary medicine and 5 technicians. One of the key conditions for designation NRL is that the laboratory is staffed by personnel with an extensive knowledge of the disease. They are familiar with the disease in the field, its epidemiology, transmission, control, clinical appearance and pathological features and are the best in understanding and performing of laboratory test results, including the assessment of accuracy and predictive value of individual tests.

Methods for CSF diagnosis are laid down in The Rulebook on the establishment of measures for early detection, diagnosis, prevention of the spread, prevention and eradication of classical swine fever as well as the way of their implementation ("Sl. glasnik RS", br. 102/2009) and are in line with EU Diagnostic manual. CSF diagnosis is based on serology, virology and molecular methods. Apparently from knowledge and skills of employees, availability of modern equipment is very important for achieving and maintaining the high quality of laboratory performing. Investments in new equipment and knowledge are the priorities of the Institute. Equipment which is in use is very modern and provided by the Ministry of Agriculture, Trade, Forestry and Water Management and EU Projects.

Methods for CSF diagnosis require using of reference materials. NRL uses reference materials provided by EURL and produce own secondary reference materials which are available for other laboratories in the country. Those materials are used for primary and secondary validations as well quality control of commercial available tests.

Accreditation of the methods according to ISO/IEC 17025 had been started in 2005 with Ab ELISA accreditation before NRL has been designated. Today, apart from Ab ELISA, Ag ELISA, virus isolation and RT-PCR are accredited methods.

For antibody detection, Ab ELISA and VNT are in use. Vaccination against CSF is still mandatory, so these tests are useful only for vaccine coverage control in domestic pigs, and detection of CSFV infection in wild boars because since January 2011 vaccination of wild boars is forbidden. Every positive Ab ELISA test in wild boars has to be checked on VNT with gt 1.1. (vaccine strains) and gt 2.3. (field strains).

For virus detection, Ag ELISA, RT-PCR, virus isolation and FAT are in use. According to the regulations, disease has to be confirmed by two positive tests. Due to the differences in tests` sensitivity and specificity, samples quality and virus characteristics, NRL has available methods for the detection of different virus structures (proteins, genome) as well as live virus - isolation. One of the main NRL tasks is maintaining and characterization of CSFV isolates. NRL has formed gene bank with the sequences of isolates from previous ten years.

The best virus characterization is possible analyzing the whole genome. Since this is not always available, sequencing and analysis are commonly done for E2, 5`NCR and NS5B regions. By the molecular analysis, it is shown that all recent isolates from Serbia belongs to gt 2.3. Confirmation of our results is done in EURL. Every new outbreak has to be confirmed in EURL in which gene bank Serbian sequences are also submitted. Such data bases are so important for molecular epidemiology, virus origin determination, and way of spreading and introducing of the virus. In the case of primary outbreak CSF diagnosis is done by real time RT-PCR and one of those tests, AgELISA, virus isolation or FAT. In the case of negative results, differential diagnosis has to be done, particularly on ASF. In the case of secondary outbreak, real time RT-PCR should be performed.

Since 2008 NRL has participated in ILCT organized by EURL. Also, NRL organizes ring trials at national level according to ISO/IEC Guide 43. Apart from NRL, in Serbia there are 11 more laboratories which are, in the case that are approved for CSF diagnosis, obliged to participate in national ring trials. In 2010, only serology tests were performed, but in 2011, tissue samples were also sent to the lab for virus detection. The both ring trials had regional and international character since Montenegro`s and Bosnian laboratories participated. About results, NRL reports to Veterinary Directorate. Depending on the results, NRL advises laboratories, organizes workshops and trainings at NRLs place or regional laboratories in order to eliminate inaccordances.

In order to keep preparedness and trained staff, NRL organizes trainings and simulation practices; the plan is to share this with other labs. NRL is particulary engaged in surveillance programs NRL in order to detect the disease as early as possible and following the movement of the disease in case when is endemic present. Very tight cooperation NRL has with Ministry and Veterinary Directorate.

Animal and animal products trade requests that veterinary authorities access with reliable, objective and deep knowledge about disease, measures for prevention of spreading and for animal health and welfare improving. NRL is a source of such knowledge and participate in directives, instructions, diagnostic manuals producing.

Apart from very responsible CSF diagnosis, researchers do investigations and public their results. Currently, NRL colaborate with FLI on two Projects, new approach to CSF control in backyard systems and evaluation of CSF marker vaccine.

Due to political and socio-economic consequences, fast and reliable diagnosis of notifiable animal diseases is of utmost importance. To ensure that results are comparable and interpretable among laboratories on national and international levels, a harmonized and up-to-date methodology is required from NRL. This can only be achieved if laboratories cooperate on different levels in a laboratory network, use methods recommended by NRL, while NRL provides them

with confirmatory testing giving that way support to the decision makers in such particular phases like Serbia is now, just before stopping of vaccination strategy.

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**NACIONALNA REFERENTNA LABORATORIJA ZA KLASIČNU KUGU
SVINJA
– ULOGA I ZNAČAJ –**

Vesna Milićević¹, Ljubiša Veljović¹, Jelena Maksimović Zorić¹, Dobrila Jakić-Dimić¹

¹ Naučni institut za veterinarstvo Srbije, Nacionalna referentna laboratorija za klasičnu kugu svinja, Vojvode Toze 14, 11000 Beograd, Srbija

Kratak sadržaj

Nacionalna referentna laboratorija za klasičnu kugu svinja je osnovana 08.7.2009. godine rešenjem Ministarstva poljoprivrede, šumarstva i vodoprivrede Republike Srbije br. 323-06-5309/2009-05 kojim su na osnovu uslova u pogledu objekta, prostorija, uređaja, odgovarajuće opreme i stručnog kadra Naučnom institutu za veterinarstvo Srbije povereni poslovi laboratorijske dijagnostike klasične kuge svinja, u skladu sa Zakonom o veterinarstvu, članovi 21 i 27. Obaveze Nacionalne referentne laboratorije koje su definisane ovim rešenjem su laboratorijska dijagnostika klasične kuge svinja u uzorcima patološkog materijala domaćih i divljih životinja, referentna laboratorijska ispitivanja, čuvanje referentnih seruma i standardnih reagenasa, izolata virusa klasične kuge svinja, uvođenje novih dijagnostičkih metoda, testiranje i provera kvaliteta vakcina i dijagnostičkih reagenasa, organizacija međulaboratorijskog uporednog testiranja laboratorija na nacionalnom nivou kao i potvrdna ispitivanja nalaza dobijenih u drugim laboratorijama.

Ključne reči: klasična kuga svinja, nacionalna referentna laboratorija

Uvod

Naučni institut za veterinarstvo Srbije (NIVS) je najstarija veterinarska institucija, osnovana 1926. godine. Osnovne delatnosti Instituta su primemenjena i razvojna istraživanja u biotehničkim naukama, klinička i laboratorijska ispitivanja u funkciji zaštite zdravlja životinja i ljudi, prevencija, suzbijanje i eradicija zaraznih bolesti životinja, ispitivanje kvaliteta hrane za ishranu životinja i namirnica animalnog porekla, ispitivanje lekova.

U odnosu na delatnost, Institut je organizovan u dva Zavoda, Zavod za kontrolu hrane i lekova i Zavod za zdravstvenu zaštitu. U zavodu za zdravstvenu zaštitu nalaze se odeljenje za virusologiju, odeljenje za imunologiju, odeljenje za bakteriologiju i parazitologiju, odeljenje za patološku morfologiju, odeljenje za opštu epizootologiju, odeljenje za epizootologiju i zdravstvenu zaštitu riba, odeljenje za epizootologiju i zdravstvenu zaštitu svinja, odeljenje za epizootologiju i zdravstvenu zaštitu živine i ptica, odeljenje epizootologiju, reprodukciju i zdravstvenu zaštitu preživara i kopitara i laboratorija za bolesti sa liste A i BSE.

Naučni institut za veterinarstvo je nacionalna referentna laboratorija za klasičnu kugu svinja, afričku kugu svinja, slinavku i šap, vezulukarnu bolest svinja,

besnilo, bolest plavog jezika, infektivnu anemiju konja, influencu konja, durinu, sakagiju, enzootsku leukozu goveda, brucelozu, salmonelozu i bolesti riba.

Nacionalne referentne laboratorije imaju ulogu u harmonizaciji laboratorijskih dijagnostičkih testova i standardizaciji veterinarskih vakcina i obezbeđivanju poverenja i međunarodnom prometu. Ove aktivnosti podrazumevaju savetodavnu i konsultantsku ulogu, naučno istraživački rad, publikacije, organizovanje treninga, nadzor nad bolestima, čuvanje kultura mikroorganizama, validaciju referentnih metoda, pripremu referentnih materijala i organizovanje međulaboratorijskih ispitivanja (Edwards i Alexander, 1998).

Nacionalna referentna laboratorija za klasičnu kugu svinja (NRL) je osnovana 08.7.2009. godine rešenjem Ministarstva poljoprivrede, šumarstva i vodoprivrede Republike Srbije br. 323-06-5309/2009-05 kojim su na osnovu uslova u pogledu objekta, prostorija, uređaja, odgovarajuće opreme i stručnog kadra Naučnom institutu za veterinarstvo Srbije povereni poslovi laboratorijske dijagnostike klasične kuge svinja, u skladu sa Zakonom o veterinarstvu, članovi 21 i 27.

Obaveze Nacionalne referentne laboratorije koje su definisane ovim Rešenjem su laboratorijska dijagnostika klasične kuge svinja u uzorcima patološkog materijala domaćih i divljih životinja, referentna laboratorijska ispitivanja, čuvanje referentnih seruma i standardnih reagenasa, izolata virusa klasične kuge svinja, uvođenje novih dijagnostičkih metoda, testiranje i provera kvaliteta vakcina i dijagnostičkih reagenasa, organizacija međulaboratorijskog uporednog testiranja laboratorija na nacionalnom nivou kao i potvrđna ispitivanja nalaza dobijenih u drugim laboratorijama.

U Nacionalnoj referentnoj laboratoriji za klasičnu kugu svinja zaposlena su 3 doktora veterinarske medicine i 5 tehničkih saradnika. Obučen i stručan kadar je prvi uslov za obavljanje poslova Nacionalne referentne laboratorije. Istraživači referentne laboratorije bave se praćenjem pojave bolesti na terenu, epidemiologijom, načinima prenošenja, kontrolom, kliničkom slikom i patologijom bolesti i najstručniji su u izvođenju i tumačenju laboratorijskih metoda dijagnostike, proceni tačnosti i prediktivnih vrednosti pojedinačnih testova.

Metode kojima se vrši dijagnostika klasične kuge svinja su definisane u Pravilniku o utvrđivanju mera za rano otkrivanje, dijagnostiku, sprečavanje širenja, suzbijanje i iskorenjivanje zarazne bolesti klasične kuge svinja, kao i načinu njihovog sprovođenja ("Sl. glasnik RS", br. 102/2009) i usklađene su sa Dijagnostičkim priručnikom Evropske unije. Dijagnostika klasične kuge svinja je bazirana na serološkim, virusološkim i molekularnim metodama.

Pored znanja i stručnosti zaposlenih, veoma važan faktor za postizanje i održavanje visokog kvaliteta rada je dostupnost savremene opreme. Ulaganja u osavremenjavanje opreme i obučenosť kadrova su prioriteti Instituta. Oprema koja se koristi je najsavremenija, zahvaljujući finansijskoj podršci Ministarstva poljoprivrede, trgovine, šumarstva i vodoprivrede Republike Srbije, odnosno Projektima sa Evropskom unijom.

Metodologija koja se koristi u Nacionalnoj referentnoj laboratoriji za klasičnu kugu svinja podrazumeva i upotrebu referentnih standarda. Nacionalna referentna laboratorija koristi referentne materijale koje Referentna laboratorija Evropske unije (EURL) obezbeđuje ali isto tako pravi i sekundarne referentne

materijale koji su dostupni ostalim laboratorijama u zemlji. Ovi materijali koriste se za primarnu i sekundarnu validaciju metoda, odnosno proveru kvaliteta komercijalno dostupnih dijagnostikuma.

Akreditacija metoda prema standardu ISO/IEC 17025 započeta je 2005. godine pre nego što je NIVS i određen za NRL, akreditacijom AbELISA testa. Danas su pored AbELISA testa akreditovane i AgELISA, izolacija virusa klasične kuge svinja na kulturi tkiva i RT-PCR.

Serološka dijagnostika klasične kuge svinja vrši se primenom AbELISA i virus neutralizacionog testa (VNT). Vakcinacija protiv klasične kuge svinja je još uvek obavezna u Srbiji, te se ovi testovi koriste za kontrolu sprovođenja vakcinacije kod domaćih svinja, odnosno za otkrivanje infekcije kod divljih svinja, s obzirom da je vakcinacija divljih svinja zabranjena od januara 2011. Pošto je prošla tek jedna godina od zabrane vakcinacije divljih svinja, svaki pozitivan nalaz na AbELISA se proverava određivanjem visine i razlike VN titrova protiv genotipova 1.1. (vakcinalni sojevi) i genotipa 2.3. (terenski sojevi koji su prethodnih godina cirkulisali teritorijom Srbije).

Za otkrivanje virusa klasične kuge svinja koriste se AgELISA, RT-PCR, izolacija virusa i test fluorescentnih antitela. U skladu sa zakonskom regulativom, potvrda izbijanja bolesti vrši se dvema metodama. Zbog različitih karakteristika testova, kvaliteta uzoraka i karakteristika virusa, NRL raspolaže metodama kojima se dokazuju različite strukture virusa (proteini, genom), odnosno dokazuje živ virus - izolacija.

Jedan od zadataka NRL je čuvanje i karakterizacija izolata virusa KKS. Nacionalna referentna laboratorija je formirala banku gena virusa klasične kuge svinja izolovanih tokom poslednje decenije na teritoriji Srbije. Za potpunu karakterizaciju virusa, idealno je sekvencionirati ceo genom. Ali kako ovo nije dostupno, sekvencioniranje i analiza se vrši na nivou regiona E2, 5'NCR i NS5B. Molekularnom analizom je utvrđeno da svi izolati virusa klasične kuge svinja iz Srbije pripadaju gt 2.3. Potvrдна ispitivanja naših rezultata vrši Referentna laboratorija Evropske unije sa kojom NRL ostvaruje veoma uspešnu saradnju. Svaki novi slučaj izbijanja bolesti takođe se potvrđuje u EURL u čijoj se banci gena nalaze i naši izolati. Ovakve baze podataka su od neprocenjivog značaja za molekularnu epidemiologiju, istraživanje porekla, načina širenja i unosa virusa kao i njegovu evoluciju.

Dijagnostika klasične kuge svinja u slučaju primarnog izbijanja vrši se real time RT-PCR testom i jednim od AgELISA, izolacijom virusa ili testom fluorescentnih antitela. U slučaju negativnog nalaza vrši se diferencijalna dijagnostika, pre svega na afričku kugu svinja. U slučaju sekundarnog izbijanja dijagnostika se vrši real time RT-PCR testom.

Od 2008. godine NRL učestvuje u ring testiranjima koje organizuje EURL čime potvrđuje svoju kompetentnost. Takođe, NRL organizuje međulaboratorijska ispitivanja na nacionalnom nivou u skladu sa ISO/IEC Guide 43. U Srbiji, pored NIVSa, postoji 11 regionalnih instituta koji su obavezni, ukoliko su ovlašćeni da rade dijagnostiku KKS, da učestvuju u međulaboratorijskim ispitivanjima koja organizuje NRL. Međulaboratorijska ispitivanja u 2010. godini obuhvatila su samo serološku dijagnostiku, a učestvovala su 4 laboratorije. U 2011. godini, pored serološke dijagnostike, laboratorijama su poslani uzorci tkiva za molekularnu

dijagnostiku. Međulaboratorijska ispitivanja koje organizuje NRL regionalnog i međunarodnog su karaktera; u ispitivanjima učestvuju laboratorije iz Crne Gore i Bosne i Hercegovine. O rezultatima međulaboratorijskih ispitivanja, NRL izveštava upravu za veterinu. Takođe, na osnovu rezultata, NRL izdaje preporuke laboratorijama učesnicama, organizuje obuke u NRL, a takođe stručnjaci NRL odlaze u regionalne laboratorije radi otklanjanja eventualnih neusaglašenosti.

Radi održavanja spremnosti i istreniranosti, NRL za svoje zaposlene organizuje redovne vežbe i simulacije pojave bolesti, što je i plan da se ove godine otpočne sa drugim laboratorijama. NRL je posebno angažovana u programima nadzora bolesti radi pouzdanog i najranijeg otkrivanja novih slučajeva izbijanja bolesti kao i kretanje bolesti kada je KKS endemski bila prisutna. S tim u vezi, najbliža saradnja je ostvarena sa Ministarstvom poljoprivrede, trgovine, šumarstva i vodoprivrede Republike Srbije, odnosno Upravom za veterinu. Trgovina životinjama i proizvodima od životinja, nametnula je vlastima veliku potrebu da raspoložu pouzdanim, objektivnim i naučno utemeljenim znanjem o samoj bolesti, merama za sprečavanje širenja, poboljšanje zdravlja i dobrobiti životinja. NRL je upravo izvor ovakvog znanja. NRL učestvuje u pisanju regulativa, uputstava, dijagnostičkih priručnika itd.

Pored veoma odgovornog pristupa dijagnostici KKS, istraživači Nacionalne referentne laboratorije se bave i istraživačkim radom o čemu svedoče mnogi objavljeni naučni i stručni radovi, magistarske i doktorske teze, monografije. Trenutno NRL ostvaruje saradnju sa Fridrih Lefler Institutom iz Nemačke kroz dva naučna projekta, koji se bave sa jedne strane novim pristupom kontrole KKS u seoskim gazdinstvima i sa druge ispitivanjem marker vakcine protiv KKS.

Imajući u vidu političke i socio-ekonomske posledice, brza i pouzdana dijagnoza klasične kuge svinja je od posebnog značaja. Uloga Nacionalne referentne laboratorije je da obezbedi pouzdane i tačne rezultate ne samo svoje već i drugih laboratorija. Prvi preduslov za ostvarivanje ovog cilja je saradnja između njih (2). Formiranje mreže laboratorija koje uspešno saraduju, u kojoj regionalne laboratorije primenjuju metode preporučene od strane NRL kao prvu liniju dijagnostike za testiranje velikog broja uzoraka, a NRL obezbeđuje potvrdna ispitivanja predstavlja realan oslonac veterinarskom sistemu, koji je naročito značajan i neophodan u fazi eradikacije u kojoj se Srbija trenutno nalazi, a pred prestanak vakcinacije protiv klasične kuge svinja, odnosno donošenje važnih odluka kako se dalje boriti protiv ove naročito opasne i veoma kontagiozne zarazne bolesti.

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